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Prostate Cancer

# A 17-gene Assay to Predict Prostate Cancer Aggressiveness in the Context of Gleason Grade Heterogeneity, Tumor Multifocality, and Biopsy Undersampling

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**Abstract**

***Background:*** Prostate tumor heterogeneity and biopsy undersampling pose challenges to accurate, individualized risk assessment for men with localized disease.
***Objective:*** To identify and validate a biopsy-based gene expression signature that predicts clinical recurrence, prostate cancer (PCa) death, and adverse pathology.
***Design, setting, and participants:*** Gene expression was quantified by reverse transcription–polymerase chain reaction for three studies—a discovery prostatectomy study ($n$ = 441), a biopsy study ($n$ = 167), and a prospectively designed, independent clinical validation study ($n$ = 395)—testing retrospectively collected needle biopsies from contemporary (1997–2011) patients with low to intermediate clinical risk who were candidates for active surveillance (AS).
***Outcome measures and statistical analysis:*** The main outcome measures defining aggressive PCa were clinical recurrence, PCa death, and adverse pathology at prostatectomy. Cox proportional hazards regression models were used to evaluate the association between gene expression and time to event end points. Results from the prostatectomy and biopsy studies were used to develop and lock a multigene-expression-based signature, called the *Genomic Prostate Score* (GPS); in the validation study, logistic regression was used to test the association between the GPS and pathologic stage and grade at prostatectomy. Decision-curve analysis and risk profiles were used together with clinical and pathologic characteristics to evaluate clinical utility.
***Results and limitations:*** Of the 732 candidate genes analyzed, 288 (39%) were found to predict clinical recurrence despite heterogeneity and multifocality, and 198 (27%) were predictive of aggressive disease after adjustment for prostate-specific antigen, Gleason

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score, and clinical stage. Further analysis identified 17 genes representing multiple biological pathways that were combined into the GPS algorithm. In the validation study, GPS predicted high-grade (odds ratio [OR] per 20 GPS units: 2.3; 95% confidence interval [CI], 1.5–3.7; $p < 0.001$) and high-stage (OR per 20 GPS units: 1.9; 95% CI, 1.3–3.0; $p = 0.003$) at surgical pathology. GPS predicted high-grade and/or high-stage disease after controlling for established clinical factors ($p < 0.005$) such as an OR of 2.1 (95% CI, 1.4–3.2) when adjusting for Cancer of the Prostate Risk Assessment score. A limitation of the validation study was the inclusion of men with low-volume intermediate-risk PCa (Gleason score 3 + 4), for whom some providers would not consider AS.

***Conclusions:*** Genes representing multiple biological pathways discriminate PCa aggressiveness in biopsy tissue despite tumor heterogeneity, multifocality, and limited sampling at time of biopsy. The biopsy-based 17-gene GPS improves prediction of the presence or absence of adverse pathology and may help men with PCa make more informed decisions between AS and immediate treatment.

***Patient summary:*** Prostate cancer (PCa) is often present in multiple locations within the prostate and has variable characteristics. We identified genes with expression associated with aggressive PCa to develop a biopsy-based, multigene signature, the *Genomic Prostate Score* (GPS). GPS was validated for its ability to predict men who have high-grade or high-stage PCa at diagnosis and may help men diagnosed with PCa decide between active surveillance and immediate definitive treatment.



## 1. Introduction

Prostate-specific antigen (PSA) screening has been associated with a decline in PCa mortality but also has led to overdiagnosis and overtreatment of biologically insignificant disease [1]. Consequently, for selected patients with low-risk disease, active surveillance (AS)—expectant management with curative intervention only for those with tumor progression—is endorsed in practice guidelines as an alternative to immediate therapy [2]. Despite these recommendations, AS is underutilized, and >90% of men diagnosed with low-risk disease receive immediate treatment with surgery or radiation [3]. Overtreatment of biologically insignificant disease results in substantial cost and unnecessary morbidity [4], leading some agencies and professional organizations to question the value of routine screening [5,6].

An important impediment to the adoption of AS is the imperfect accuracy of conventional risk assessment at initial diagnosis [7]. Pretreatment risk-assessment tools [8,9] based on PSA, clinical stage, Gleason score, and other biopsy characteristics fare well in identifying patients at risk of aggressive disease but predict indolent disease for only a limited proportion of patients [10,11]. Moreover, for a substantial proportion (20–60%) of men classified as low-risk, current pretreatment assessment tools underestimate true tumor grade and, less commonly, true stage [12–14].

Molecular analyses of localized PCa have enabled the investigation of prognostic markers including tissue-based gene expression signatures, systems pathology profiles, and urine-based molecular markers [15,16]. Although many groups have demonstrated the potential of gene expression analysis to predict outcome in localized PCa [17–20], frequent genetic differences between regions of individual tumors and limited tumor sampling by needle biopsy pose challenges to molecular-based assays in PCa [21,22]. With these challenges in mind, we conducted two studies to identify genes for which expression in both prostatectomy and biopsy tissues consistently correlates with tumor aggressiveness regardless of multifocality, heterogeneity, or technical challenges associated with limited tumor obtained through biopsy. We then performed a third, independent, clinical validation study to determine whether a prespecified 17-gene signature can be measured in prostate biopsies to predict adverse pathology and improve risk stratification at diagnosis.

## 2. Materials and methods

### 2.1. Study design, patients, and specimens

Three studies were performed and are referred to as the *prostatectomy study*, the *biopsy study*, and the *validation study* (Fig. 1A; Supplement). The prostatectomy study sampled from a cohort of 2641 clinical T1/T2 PCa patients treated by radical prostatectomy at the Cleveland Clinic from 1987 to 2004. All patients with clinical recurrence (local recurrence or distant metastasis, $n = 127$) were selected, together with a random sampling of nonrecurrent patients, using an established stratified cohort sampling method ($n = 374$, with a 1:3 ratio of recurrent to non-recurrent patients) [23,24]. All samples analyzed were from fixed paraffin-embedded (FPE) prostatectomy specimens. The biopsy study included FPE prostate needle biopsy specimens from a separate cohort of 167 patients who had a diagnostic biopsy and underwent prostatectomy within 6 mo of diagnosis at the Cleveland Clinic between 1999 and 2007. Disease and vital status were determined from a database that was maintained prospectively, approved by an institutional review board (IRB), and compliant with the US Health Insurance Portability and Accountability Act, using data updated through October 2008.

The validation study conformed to the REMARK (Reporting Recommendations for Tumor Marker Prognostic Studies) guidelines for biomarker validation [25]. This prospectively designed protocol, including gene panel, algorithm, end points, analytical methods, and statistical methods, was agreed to by all investigators and locked prior to analyses. Consenting patients were identified from the IRB-approved University of California San Francisco (UCSF) Helen Diller Family Comprehensive Cancer Center Urologic Oncology Data Base (UODB). Men included were potential candidates for surveillance [26] but elected prostatectomy within 6 mo of their initial diagnostic biopsies (additional details are provided in the Supplement).